

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) ~~An~~ The isolated or purified enzyme of claim 27 ~~exhibiting nicotianamine synthase activity~~, wherein the enzyme comprises the polypeptide having an amino acid sequence of SEQ ID NO: 1.

2. (Canceled)

3. (Previously Presented) The enzyme according to claim 27, wherein the enzyme comprises the consensus amino acid sequence of ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27).

4. – 26. (Canceled)

27. (Currently Amended) An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein the enzyme comprises a polypeptide that is at least 90% identical to SEQ ID NO:1.

a. ~~is a polypeptide having at least 50% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:~~

(1) ~~₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)~~

(2) ~~₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)~~

(3) ~~₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)~~

(4) ~~₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)~~

(5) ~~₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)~~

(6) ~~₂₅₃RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28); and~~

b. ~~has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.~~

28. (Previously Presented) The enzyme of claim 27, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

29. (Canceled)

30. (Previously Presented) The enzyme of claim 27, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.

31. (Previously Presented) The enzyme of claim 27, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

32. (Canceled)

33. (Previously Presented) The enzyme of claim 27, wherein the enzyme is isolated or purified from barley.

34. (Canceled)

35. (Previously Presented; Allowable) A mutated enzyme exhibiting nicotianamine synthase activity, wherein the enzyme:

a. is a polypeptide having more than 95% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:

(1) ₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)

(2) ₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)

(3) ₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)

(4) ₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)

(5) ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)

(6) ₂₅₃RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28); and

b. has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

36. (Previously Presented; Allowable) The enzyme of claim 35, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203), A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

37. (Previously Presented; Allowable) The enzyme of claim 35, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

38. (Previously Presented; Allowable) The enzyme of claim 35, wherein the polypeptide has more than 97% identity with an amino acid sequence of SEQ ID NO: 1.

39. (Previously Presented) The enzyme of claim 27, wherein the polypeptide has more than 97% identity with an amino acid sequence of SEQ ID NO: 1.

40. (Canceled)

41. (Previously Presented; Allowable) An isolated, purified, or mutated enzyme exhibiting nicotianamine synthase activity, wherein the enzyme comprises an active fragment of an amino acid sequence of SEQ ID NO: 1, the active fragment comprising a polypeptide, wherein the polypeptide:

a. comprises at least one consensus sequence of SEQ ID NO: 1 that is:

(1) ₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)

(2) ₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)

(3) ₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)

(4) ₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)

(5) ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)

(6) ₂₅₃RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28); and

b. has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

42. (Cancel)

43. (Previously Presented) An isolated or purified barley enzyme exhibiting nicotianamine synthase activity, wherein:

a. the enzyme is:

i. isolated or purified from barley; or

- ii. expressed directly or indirectly from a nucleic acid isolated or purified from barley; or
 - iii. expressed directly or indirectly from a chimeric nucleic acid at least partially isolated or purified from barley;
- b. the enzyme comprises a polypeptide having at least ~~50~~90% identity with an amino acid sequence of SEQ ID NO: 1, comprising at least one consensus sequence of SEQ ID NO: 1 that is:
- (1) ₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)
 - (2) ₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)
 - (3) ₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)
 - (4) ₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)
 - (5) ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)
 - (6) ₂₅₃RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28); and
- c. the enzyme has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

44. (Previously Presented) The enzyme of claim 43, wherein the polypeptide further comprises all of the conserved amino acid residues of SEQ ID NO: 1 that is:

L(11), K(14), I(15), I(22), L(25), L(28), P(30), L(37), F(38), L(41), V(42), C(45), P(47), D(52), V(53), Q(61), M(63), R(64), L(67), I(68), C(71), A(74), E(75), L(78), E(79), H(81), L(86), D(90), P(92), L(93), H(95), L(96), F(99), P(100), Y(101), N(104), Y(105), L(108), L(111), E(112), L(115), L(116), A(129), F(130), G(132), S(133), G(134), P(135), L(136), P(137), S(140), L(143), A(144), H(147), L(148), F(153), N(155), A(162), N(163), A(166), L(169), R(180), M(181), F(183), T(185), L(195), D(199), V(200), V(201), F(202), L(203),

A(204), A(205), V(207), G(208), M(209), K(214), H(220), L(221), H(224), M(225), G(228), A(229), L(231), R(239), F(241), L(242), Y(243), P(244), V(246), G(255), F(256), V(258), L(259), V(261), H(263), P(264), V(268), N(270), S(271), K(277) (SEQ ID NO: 29).

45 (Previously Presented). The enzyme of claim 43, wherein the polypeptide has more than 90% identity with an amino acid sequence of SEQ ID NO: 1.

46 (Previously Presented). The enzyme of claim 43, wherein the polypeptide has more than 95% identity with an amino acid sequence of SEQ ID NO: 1.

47 (Previously Presented). The enzyme of claim 43, wherein the nicotianamine synthase activity is measured in an assay in a comparison with the enzyme of SEQ ID NO:1.

48-57. (Cancel)

58. (New) An isolated or purified enzyme exhibiting nicotianamine synthase activity, wherein the enzyme consists of the polypeptide set forth as SEQ ID NO:1.

59. (New) The isolated or purified enzyme of claim 27, comprising at least one consensus sequence of SEQ ID NO: 1 selected from the group consisting of:

(1) ₂₅LPXLSPSPXVDRLFTXLVXACVPXSPVDVTKL₅₆ (SEQ ID NO: 23)

(2) ₆₇LIRLCSXAEGXLEAHY₈₂ (SEQ ID NO: 24)

(3) ₉₂PLDHLGXFPY₁₀₁ (SEQ ID NO: 25)

(4) ₁₂₈VAFXGSGPLPFSS₁₄₀ (SEQ ID NO: 26)

(5) ₁₉₉DVVFLAALVGM₂₀₉ (SEQ ID NO: 27)

(6) ₂₅₃RGGFXVLAVXHP₂₆₄ (SEQ ID NO: 28); and

b. has more than 25% of the relative nicotianamine synthase activity of the enzyme of SEQ ID NO:1.